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Nitric Oxide Sensors obtained through the entrapment of iron complexes in sol-gel matrix

Juliana C. Biazotto, João F. Borin, Roberto Mendonça Faria¹ and Carlos F.O Graeff
Departamento de Física e Matemática-FFCLRP-USP, Av. Bandeirantes 3900, 14040-901
Ribeirão Preto, Brazil
1-Instituto de Física de São Carlos-USP, C.P. 369, 13560-970 São Carlos, Brazil

ABSTRACT

Iron(III)-diethyldithiocarbamate (Fe3DETC) or iron(III)-tetra-pentafluorophenyl porphyrin (FeTFPP) was entrapped within a silica matrix by the sol-gel process. The obtained sol-gel materials SGFeDETC and SGFeTFPP were investigated as sensors for nitric oxide (NO). UV/Vis spectra of the SGFeTFPP present a Soret band at 410 nm similar to that found in the solution. The binding of gaseous NO resulted in a red shift in the Soret absorption band (410 to 419 nm) of the FeTFPP in the matrix unlike FeTFPP:NO in solution. In the case of SGFeDETC, after addition of sodium dithionite solution and bubbling NO we have good evidence that the complex is formed. The EPR spectrum of the SGFeDETC:NO in solid form exhibited a signal similar to that found in a solution of FeDETC:NO at 77K. The UV/Vis spectrum of SGFeDETC:NO shows a band at 367 nm also found in FeDETC:NO solutions. It is observed that the FeDETC:NO is more stable entrapped in the sol-gel than in aqueous solution. In the former the EPR signal decreases by a factor of 4 after one week, in the latter in 2 days the EPR signal cannot be observed anymore.

INTRODUCTION

Nitric oxide (NO) is an important signaling molecule that acts in many tissues to regulate a diverse range of physiological processes including neurotransmission and immune defense [1,2]. Several methods for NO detection have been developed and one of the most powerful methods for directly measuring NO production in biological systems is electron paramagnetic resonance (EPR) spin trapping techniques [3]. Iron complexes with dithiocarbamates and porphyrins are used as spin traps due to the high affinity between NO and the iron complexes, however these complexes are unstable for long-term measurements. The physical entrapment of molecules using the sol-gel process [4,5] has been used for the development of NO electrochemical and optical sensors. Sensors entrapped in gels offer numerous advantages when compared with liquid based systems: they are easier to manipulate, allow species detection and concentrations measurements with less contamination of the sample, can be used for continuous sensing, and are normally more stable. In the case of silica gels a further advantage is that it is transparent in the UV/visible/near infrared range, which makes this material especially interesting for optical sensors. For EPR measurement of special interest is the fact that solutions are in general hard to measure since liquids have high dielectric losses, or in other words they absorb microwaves thus killing the Q of the resonance cavity. EPR quartz liquid cells are necessary when liquids are to be measured by EPR, which are expensive and impose restrictions in what concerns the concentration of paramagnetic species. Thus by entrapping the paramagnetic species in a solid is of great interest for EPR. In this work we report the synthesis of the NO sensors, SGFeDETC and SGFeTFPP, which consist in the entrapment of the spin

trappings iron(III)-diethyldithiocarbamate (Fe3DETC) or iron(III)-tetra-pentafluorophenylporphyrin (FeTFPP), within an inorganic matrix by the sol-gel process.

EXPERIMENTAL DETAILS

Synthesis of SGFeDETC. Tetraethyl orthosilicate (Aldrich) (TEOS, 4,00 mL), ethanol (4,00 mL) and concentrated HCl (150 μ L) was sonicated for 10 min. After that, Triton X100 (VETEC) (50 μ L) was added to a silica sol and the resultant solution added to the Fe3DETC (Aldrich) in dimethylformamide (DMF) (ratio DETC:Fc, 2:1). The mixture was maintained under stirring for 15 hours and allowed to stand at 30° C for aging.

Synthesis of SGFeTFPP. TEOS (4,00 mL), ethanol (4,00 mL), HCl 0,1 M (50,0 μ L), water (310 μ L) and Triton X100 (40,0 μ L) was sonicated for 10 min. The resultant solution was added to the FeTFPP (Midcentury) in dichloromethane (DCM). The mixture was maintained under stirring for 2 hours and allowed to stand at 30° C for aging.

NO generation. Gaseous NO was obtained in the reaction of metallic copper and aqueous solution of HNO₃ and passed through a trap containing concentrated NaOH solution.

Preparation of the iron nitrosyl SGFeDETC:NO. Sodium dithionite (Na₂S₂O₃) was added to a piece of SGFeDETC in water. After 10 min. the material was removed, added to a saturated aqueous NO solution and allowed to stand for 5 minutes.

UV/Vis spectra (Varian, Cary 50 spectrophotometer) were recorded using a 0.2-cm path length quartz cell with a grounded material in DCM. For sol-gel iron nitrosyl complexes, NO gas was bubbled in a suspension of grounded sol-gel materials and degassed DCM.

EPR (computer interfaced Varian E-4 X-Band spectrometer) was performed using a quartz tube and a cryostat for measurements at 77K and flat cell at room temperature.

RESULTS AND DISCUSSION

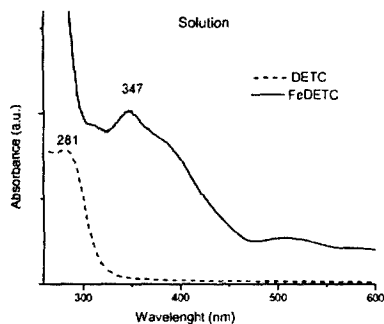


Figure 1. Absorption spectra of Fe3DETC and DETC in DCM.

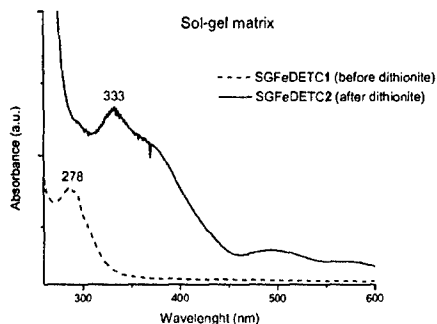


Figure 2. Absorption spectra of SGFeDETC1 and SGFeDETC2 in DCM suspension.

The sol-gel materials containing Iron(III)-diethyldithiocarbamate (Fe3DETC) and iron(III)-tetra-pentafluorophenylporphyrin were done by adding a polymeric mixture (colloidal) to an iron (III) complexes solution. The gelation process involves the hydrolysis and condensation of ethoxyl groups of TEOS to generate siloxane bonds in the gel [6]. The evaporation of the solvents led to a dry solid with a glassy appearance containing the iron-complexes caged into the pores of the silica matrix.

Fe3DETC in DMF is brown in color but after addition of the colloidal solution and stirring about 3 hours its color changes to clear yellow and remains in the final glass material. Figure 1 shows the UV/Vis spectra of the Fe3DETC and DETC in solution and Figure 2 present the spectra of SGFeDETC before (SGFeDETC1) and after (SGFeDETC2) sodium dithionite. We observed that Fe^{3+} complex exhibits three absorption bands at 347, 385 and 510 nm [7] while SGFeDETC1 a band at 278 nm. Therefore, the spectra are not similar and indicate that the iron (III) complex is formed just after the addition of sodium dithionite. A possible explanation is that the protonation of the NCS_2^- group of the complex $(\text{CH}_3\text{CH}_2)_2\text{NCS}_2^--\text{Fe}^{3+}--\text{S}_2\text{NC}(\text{CH}_2\text{CH}_3)_2$ through the HCl present in the medium, leads to the iron decomplexation. In fact, SGFeDETC1 spectrum is similar to the DETC spectrum with bands at 278 and 281 nm respectively. With addition of a reducing agent, dithionite solution, to SGFeDETC1, the glass gradually changed its color to brown and the spectrum (Fig. 2) became similar to that Fe3DETC solution (Fig. 1). Thus, probably the reducing agent removed the proton of NCSH groups restoring the anion, which than can complex with Fe^{3+} , regenerating the Fe3DETC inside of the matrix. Nevertheless, it was observed a 14 nm blue shift in the optical absorption bands of the SGFeDETC2 with respect to the one found in solution, which can be attributed to changes of the molecule geometry induced by the rigid environments [8].

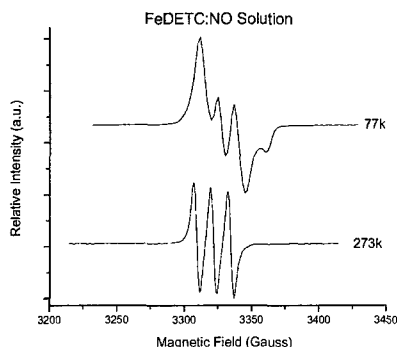


Figure 3. EPR spectra of the FeDETC:NO in DMF at 273K and 77K. Microwave power of 20 mW and modulation amplitude of 1.6 G.

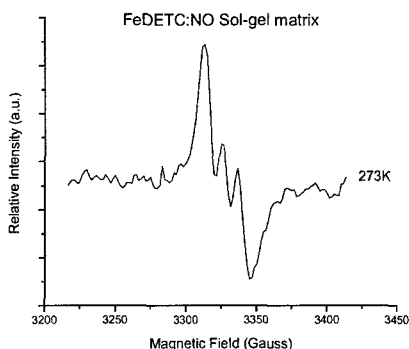


Figure 4. EPR spectra of the SGFeDETC2:NO at 273K. Microwave power of 20 mW and modulation amplitude of 1.6 G.

Although only Fe2DETC:NO shows a characteristic signal in the EPR spectrum, it was reported that diamagnetic Fe3DETCNO is gradually converted into paramagnetic Fe2DETC:NO [7]. Likewise we observed that by exposing the SGFeDETC1 to dithionite and NO, the glass changes its color from brown to green, which is the characteristic color of the iron

nitrosylcomplex. Figure 3 displays the EPR spectra of the FeDETC:NO solution (room temperature and 77K) and Figure 4 SGFeDETC2:NO spectrum at room temperature.

It's interesting to note that EPR spectrum of the FeDETC:NO in solution gave a characteristic three line signal at room temperature ($g = 2,035$) while ironnitrosyl complex caged in the matrix exhibit an EPR signal analogous to that FeDETC:NO at 77K (axial symmetry). This result indicates, in good agreement with the UV/VIS observations that the rigid environment of the silica matrix has important effects on the electronic properties of FeDETC:NO. The fact that the complex in the gel behaves like the freeze solution is a clear indication that the molecule rotations are hindered when encaged.

Further evidence for the formation of FeDETC:NO complex inside the gel can be obtained from the UV/VIS spectra. In Figure 5 the absorption spectrum of FeDETC:NO in solution, in the colloid as well as in the glass are shown. Notice the bands at 318, ~374 (more intense) and 498 nm are present in all spectra, however for the glass the band at ~367 nm is more prominent.

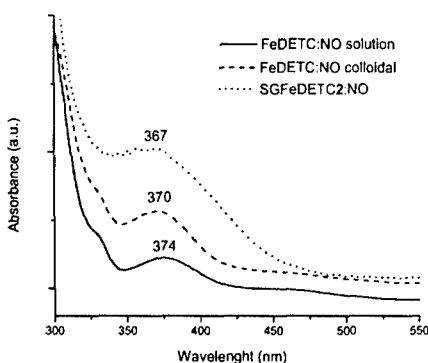


Figure 5. Optical absorption spectra of FeDETC:NO solution, FeDETC:NO colloidal and SGFeDETC2:NO (after addition of dithionite and NO).

The stability of the SGFeDETC2:NO is enhanced when compared to FeDETC:NO solutions. As can be seen in Figure 6 the EPR signal found in SGFeDETC2:NO can be measured even after seven days, with a decrease of a factor 4 in its intensity. On the other hand the EPR signal of FeDETC:NO aqueous solution kept at room temperature, is absent after two days, which means a decrease by at least a factor of 20 in the period. In fact the behavior of the SGFeDETC2:NO EPR signal is more complex, it increases at first, followed by a decrease. The increase in stability is not clearly understood, however it may be related to the rigidity of the complex environment as revealed by the EPR spectra, see figure 4. A more comprehensive discussion of the dynamics of NO in this hybrid material shall be discussed elsewhere.

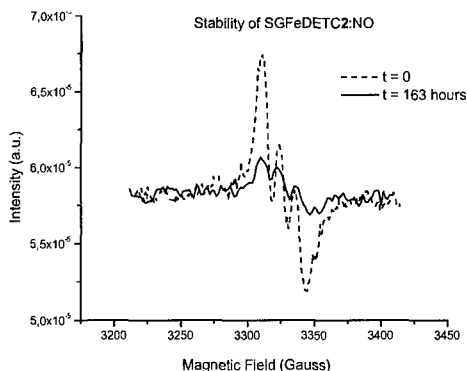


Figure 6. EPR spectra of the SGFeDETC2NO in $t = 0$ and after $t = 163$ hours. Microwave power of 20 mW and modulation amplitude of 1.6 G at 273K.

The absorption spectra of FeTFPP in solution and in sol-gel matrix are shown in Figure 7 and Figure 8, respectively. No changes in the band at 355 nm, Soret band (411 nm) and Q-band (509 nm) were observed for FeTFPP entrapped in the sol-gel matrix compared to the solution. Upon addition of NO, the Soret absorption of FeTFPP solution is red shifted to 424 nm and the band at 540 nm replaces that one at 509 nm. For SGFeTFPP:NO we observed a smaller displacement of the Soret band ($411 \rightarrow 419$ nm) than in solution (Fig. 8) and the appearance of the band at 540 nm. This suggests that either a change in the binding affinity occurred due the entrapment or that the concentration of NO we have used was not enough to saturate the Fe complex inside the sol-gel material.

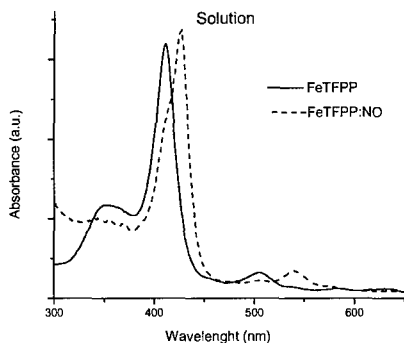


Figure 7. Optical absorption spectra of FeTFPP and FeTFPP:NO in DCM.

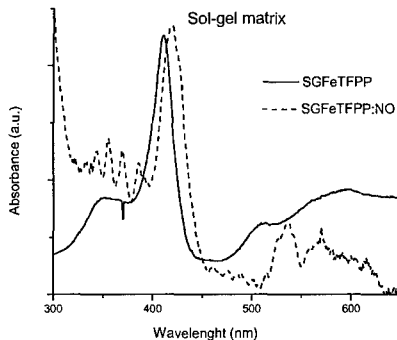


Figure 8. Optical absorption spectra of SGFeTFPP and SGFeTFPP:NO in DCM.

Notice that the changes in the band at 355 nm are inconclusive due to the fact that NO₂ molecules also absorb in this same region of the spectrum [9]. Thus it is hard to separate the effects of NO to this band, since we certainly have NO₂ in our sample too.

CONCLUSIONS

A new solid material containing the spin trap FeDETC entrapped in the silica matrix was obtained with interesting characteristics for EPR applications. The formation of this new hybrid material was done by adding FeDETC to the silica colloidal solution. Nevertheless it was found from UV/Vis analysis that in this stage the iron decomplexes probably due to acidic medium. It is necessary to add a reducing agent, in our case sodium dithionite, to restore the iron complex inside the matrix. Both SGFeTFPP and SGFeDETC can be used to bind NO as observed by UV/Vis and EPR results. SGFeDETC after NO binding gave, at room temperature, an EPR signal, which was found to be more similar to that in FeDETC:NO solutions at 77K than at room temperature. This effect was attributed to the rigid environment found in the gel, which changes the electronic properties of the complex. The stability of the FeDETC:NO complex was enhanced when entrapped. It was observed that SGFeDETC:NO can be kept at room temperature for many days with a small effect in the EPR signal, contrary to what is found in the solution. The results demonstrated that the hybrid material comprised of the sol-gel and spin traps are promising for NO EPR based or optical sensors.

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